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 NEWS  1          Web Page URLs for STN Seminar Schedule - N. America
 NEWS  2          "Ask CAS" for self-help around the clock
 NEWS  3  May 12  EXTEND option available in structure searching
 NEWS  4  May 12  Polymer links for the POLYLINK command completed in REGISTRY
 NEWS  5  May 27  New UPM (Update Code Maximum) field for more efficient patent
                  SDIs in CAplus
 NEWS  6  May 27  CAplus super roles and document types searchable in REGISTRY
 NEWS  7  Jun 28  Additional enzyme-catalyzed reactions added to CASREACT
 NEWS  8  Jun 28  ANTE, AQUALINE, BIOENG, CIVILENG, ENVIROENG, MECHENG,
                  and WATER from CSA now available on STN(R)
 NEWS  9  Jul 12  BEILSTEIN enhanced with new display and select options,
                  resulting in a closer connection to BABS

 NEWS EXPRESS  MARCH 31 CURRENT WINDOWS VERSION IS V7.00A, CURRENT
               MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
               AND CURRENT DISCOVER FILE IS DATED 26 APRIL 2004
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 NEWS INTER    General Internet Information
 NEWS LOGIN    Welcome Banner and News Items
 NEWS PHONE    Direct Dial and Telecommunication Network Access to STN
 NEWS WWW      CAS World Wide Web Site (general information)

Enter NEWS followed by the item number or name to see news on that
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FILE 'HOME' ENTERED AT 19:54:54 ON 28 JUL 2004

=> file medline biosis embase scisearch caplus wpid
COST IN U.S. DOLLARS                            SINCE FILE      TOTAL
                                                   ENTRY    SESSION
FULL ESTIMATED COST                                 0.21       0.21

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FILE 'BIOSIS' ENTERED AT 19:55:20 ON 28 JUL 2004
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FILE 'EMBASE' ENTERED AT 19:55:20 ON 28 JUL 2004
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FILE 'SCISEARCH' ENTERED AT 19:55:20 ON 28 JUL 2004
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FILE 'CAPLUS' ENTERED AT 19:55:20 ON 28 JUL 2004
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FILE 'WPIDS' ENTERED AT 19:55:20 ON 28 JUL 2004
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=> s (mutat (s) (subunit or domain)) and ((increas or more or great? or enhanc? or
potentiat?) (s) (?toxi? or poison? or lethal?))
OR IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> s (mutat (s) (subunit or domain)) (p) ((increas or more or great? or enhanc? or
potentiat?) (s) (?toxi? or poison? or lethal?))
   2 FILES SEARCHED...
   3 FILES SEARCHED...
L1            2 (MUTAT (S) (SUBUNIT OR DOMAIN)) (P) ((INCREAS OR MORE OR GREAT?
                OR ENHANC? OR POTENTIAT?) (S) (?TOXI? OR POISON? OR LETHAL?))

=> t ti l1 1-2

L1   ANSWER 1 OF 2  SCISEARCH  COPYRIGHT 2004 THOMSON ISI on STN
TI   Mutations in the diastrophic dysplasia sulfate transporter (DTDST) gene
     (SLC26A2): 22 Novel mutations, mutation review, associated skeletal
     phenotypes, and diagnostic relevance

L1   ANSWER 2 OF 2  SCISEARCH  COPYRIGHT 2004 THOMSON ISI on STN
TI   Phenotype-genotype relationships in PEX10-deficient peroxisome biogenesis
     disorder patients


=> s (mutat (s) (subunit or domain)) (p) ((increas? or more or great? or enhanc? or
potentiat?) (s) (?toxi? or poison? or lethal?))
   3 FILES SEARCHED...
L2            4 (MUTAT (S) (SUBUNIT OR DOMAIN)) (P) ((INCREAS? OR MORE OR GREAT?
                OR ENHANC? OR POTENTIAT?) (S) (?TOXI? OR POISON? OR LETHAL?))

=> dup rem
ENTER L# LIST OR (END):l2
PROCESSING COMPLETED FOR L2
L3            3 DUP REM L2 (1 DUPLICATE REMOVED)

=> l3 not l1
L3 IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> s l3 not l1
L4            1 L3 NOT L1

=> t ti l4

L4   ANSWER 1 OF 1  EMBASE  COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED.
     on STN
TI   Statistical analysis of in vivo rodent micronucleus assay.
```

```
=> s (insecticide or herbicide or pesticide) and (mutat (w) (subunit or domain))
(W) IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> s (insecticide or herbicide or pesticide) and (mutat (s) (subunit or domain))
L5            0 (INSECTICIDE OR HERBICIDE OR PESTICIDE) AND (MUTAT (S) (SUBUNIT
                OR DOMAIN))

=> s (insecticide or herbicide or pesticide) and (mutat (p) (subunit or domain))
L6            0 (INSECTICIDE OR HERBICIDE OR PESTICIDE) AND (MUTAT (P) (SUBUNIT
                OR DOMAIN))

=> s insecticide or herbicide or pesticide
L7       407169 INSECTICIDE OR HERBICIDE OR PESTICIDE

=> s  (mutat (p) (subunit or domain))
L8           72 (MUTAT (P) (SUBUNIT OR DOMAIN))

=> s (insecticide or herbicide or pesticide) and (mutat? (p) (subunit or domain))
L9          389 (INSECTICIDE OR HERBICIDE OR PESTICIDE) AND (MUTAT? (P) (SUBUNIT
                 OR DOMAIN))

=> d scan

L9    389 ANSWERS  BIOSIS  COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI    Secondary structure prediction of acetolactate synthase protein in
      sulfonylurea herbicide resistant Limnophila sessiliflora.
IT    Methods & Equipment
         gene cloning: genetic techniques, laboratory techniques; gene
         sequencing: genetic techniques, laboratory techniques
IT    Miscellaneous Descriptors
         biotype; herbicide resistance

HOW MANY MORE ANSWERS DO YOU WISH TO SCAN? (1):2

L9    389 ANSWERS  BIOSIS  COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI    Mutagenesis studies on the sensitivity of Escherichia coli
      acetohydroxyacid synthase II to herbicides and valine.

L9    389 ANSWERS  BIOSIS  COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI    Molecular biology of insecticide resistance.
IT    Miscellaneous Descriptors
         ACETYLCHOLINESTERASE; CARBAMATES; CYCLODIENES; CYTOCHROME P450;
         DETOXIFICATION; GAMMA-AMINOBUTYRIC ACID; GLUTATHIONE-S-TRANSFERASE;
         INSECTICIDES; ORGANOPHOSPHORUS COMPOUNDS; POINT MUTATIONS; PYRETHROIDS

HOW MANY MORE ANSWERS DO YOU WISH TO SCAN? (1):none

=> d his\
'HIS\' IS NOT A VALID FORMAT
In a multifile environment, a format can only be used if it is valid
in at least one of the files.  Refer to file specific help messages
or the STNGUIDE file for information on formats available in
individual files.
REENTER DISPLAY FORMAT FOR ALL FILES (FILEDEFAULT):end

=> d his

     (FILE 'HOME' ENTERED AT 19:54:54 ON 28 JUL 2004)
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     FILE 'MEDLINE, BIOSIS, EMBASE, SCISEARCH, CAPLUS, WPIDS' ENTERED AT
     19:55:20 ON 28 JUL 2004
L1             2 S (MUTAT (S) (SUBUNIT OR DOMAIN)) (P) ((INCREAS OR MORE OR GREA
L2             4 S (MUTAT (S) (SUBUNIT OR DOMAIN)) (P) ((INCREAS? OR MORE OR GRE
L3             3 DUP REM L2 (1 DUPLICATE REMOVED)
L4             1 S L3 NOT L1
L5             0 S (INSECTICIDE OR HERBICIDE OR PESTICIDE) AND (MUTAT (S) (SUBUN
L6             0 S (INSECTICIDE OR HERBICIDE OR PESTICIDE) AND (MUTAT (P) (SUBUN
L7        407169 S INSECTICIDE OR HERBICIDE OR PESTICIDE
L8            72 S  (MUTAT (P) (SUBUNIT OR DOMAIN))
L9           389 S (INSECTICIDE OR HERBICIDE OR PESTICIDE) AND (MUTAT? (P) (SUBU

=> s (increas? or more or great? or enhanc? or potentia?) (s) (?toxi? or poison? or
lethal?)
   3 FILES SEARCHED...
L10      920196 (INCREAS? OR MORE OR GREAT? OR ENHANC? OR POTENTIA?) (S) (?TOXI?
                OR POISON? OR LETHAL?)

=> s l10 and 19
L11           38 L10 AND L9

=> dup rem
ENTER L# LIST OR (END):l11
PROCESSING COMPLETED FOR L11
L12           29 DUP REM L11 (9 DUPLICATES REMOVED)

=> d scan

L12  29  ANSWERS  WPIDS  COPYRIGHT 2004 THOMSON DERWENT on STN

AN   2003-803620 [75]   WPIDS
TI   A method of post-translationally modified recombinant glycoprotein with
     properties that mimic native bioscavenger molecule to be used as human
     treatments to protect against toxicity resulting chemical/biological agent
     toxins or drugs.

HOW MANY MORE ANSWERS DO YOU WISH TO SCAN? (1):2

L12  29 ANSWERS  SCISEARCH  COPYRIGHT 2004 ISI on STN
AN   2003:684817  SCISEARCH
GA   The Genuine Article (R) Number: 705PD
TI   Genotoxicity of methoxyphosphinyl insecticide in mammalian cells
CC   ZOOLOGY
ST   Author Keywords: SCE; chromosome aberration; gene mutation;
     organophosphorus insecticide
STP  KeyWords Plus (R): HAMSTER OVARY CELLS; SISTER-CHROMATID EXCHANGES;
     INDEPENDENT GENETIC-LOCI; INDUCTION; INVITRO; TRANSFORMATION; RESPONSES;
     ACEPHATE; DNA
     *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

L12  29  ANSWERS  WPIDS  COPYRIGHT 2004 THOMSON DERWENT on STN

AN   2001-235196 [24]   WPIDS
TI   Drosophila melanogaster Bioamine Transporter 1 (BT1) nucleic acid and
     protein, useful in screening assays to identify candidate  compounds which
     are potential pesticide agents or therapeutics that interact
     with BT1 proteins.

HOW MANY MORE ANSWERS DO YOU WISH TO SCAN? (1):end

=> t ti l12 1-29
```

L12 ANSWER 1 OF 29 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
TI Genetically engineering plant-derived nucleic acid sequence encoding protein e.g., glutathione-S-transferase, that detoxifies toxin e.g., fluorodifen, by performing gene shuffling and selective mutagenesis of the nucleic acid sequences.

L12 ANSWER 2 OF 29 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
TI A method of post-translationally modified recombinant glycoprotein with properties that mimic native bioscavenger molecule to be used as human treatments to protect against toxicity resulting chemical/biological agent toxins or drugs.

L12 ANSWER 3 OF 29 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI Introduction of Culex toxicity into Bacillus thuringiensis Cry4Ba by protein engineering.

L12 ANSWER 4 OF 29 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
TI Genotoxicity of methoxyphosphinyl **insecticide** in mammalian cells

L12 ANSWER 5 OF 29 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
TI Effects of mutations of a glutamine residue in loop D of the α7 nicotinic acetylcholine receptor on agonist profiles for neonicotinoid insecticides and related ligands.

L12 ANSWER 6 OF 29 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
TI Drosophila melanogaster Bioamine Transporter 1 (BT1) nucleic acid and protein, useful in screening assays to identify candidate compounds which are potential **pesticide** agents or therapeutics that interact with BT1 proteins.

L12 ANSWER 7 OF 29 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
TI New tandem pore domain weak inward rectifying potassium ion (TWIK) channel nucleic acids and proteins, useful in assays for identifying candidate compounds which are potential pesticides or therapeutics.

L12 ANSWER 8 OF 29 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
TI A phenylalanine residue at segment D3-S6 in Nav1.4 Voltage-gated Na(+) channels is critical for pyrethroid action.

L12 ANSWER 9 OF 29 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
TI Molecular analysis of kdr-like resistance in permethrin-resistant strains of head lice, Pediculus capitis

L12 ANSWER 10 OF 29 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
TI Analyzing Caenorhabiditis elegans insulin-like gene expression, nucleic acids and proteins of the C. elegans insulin-like genes.

L12 ANSWER 11 OF 29 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
TI Insecticidal Bacillus thuringiensis proteins.

L12 ANSWER 12 OF 29 CAPLUS COPYRIGHT 2004 ACS on STN
TI Analysis of mutations in the pore-forming region essential for insecticidal activity of a Bacillus thuringiensis δ-endotoxin

L12 ANSWER 13 OF 29 CAPLUS COPYRIGHT 2004 ACS on STN
TI Identification of residues in domain III of Bacillus thuringiensis Cry1Ac toxin that affect binding and toxicity

L12 ANSWER 14 OF 29 MEDLINE on STN DUPLICATE 1
TI The pharmacological flexibility of the insect voltage gated sodium channel: toxicity of AaIT to knockdown resistant (kdr) flies.

L12 ANSWER 15 OF 29 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
TI Altered properties of neuronal sodium channels associated with genetic resistance to pyrethroids

L12 ANSWER 16 OF 29 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI Binding of Bacillus thuringiensis Cry1Ac toxin to Manduca sexta aminopeptidase-N receptor is not directly related to toxicity.

L12 ANSWER 17 OF 29 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN DUPLICATE 2
TI **Mutations** of loop 2 and loop 3 residues in **domain** II of Bacillus thuringiensis cry1c δ-endotoxin affect insecticidal specificity and initial binding to Spodoptera littoralis and Aedes aegypti midgut membranes.

L12 ANSWER 18 OF 29 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
TI Evolution of whole cells and organisms by recursive DNA sequence recombination in cells to evolve cells having acquired desired function, useful in methods for predicting the efficacy of a drug in treating viral or pathogenic infections.

L12 ANSWER 19 OF 29 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
TI Characterization and comparative pharmacological studies of a functional γ-aminobutyric acid (GABA) receptor cloned from the tobacco budworm, Heliothis virescens (Noctuidae:Lepidoptera).

L12 ANSWER 20 OF 29 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
TI Protein engineering of Bacillus thuringiensis δ- **endotoxin: Mutations** at **domain** II of CryIAb **enhance** receptor affinity and **toxicity** toward gypsy moth larvae.

L12 ANSWER 21 OF 29 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
TI Mutagenesis of three surface-exposed loops of a Bacillus thuringiensis insecticidal toxin reveals residues important for toxicity, receptor recognition and possibly membrane insertion.

L12 ANSWER 22 OF 29 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI Functional significance of loops in the receptor binding domain of Bacillus thuringiensis CryIIIA delta-endotoxin.

L12 ANSWER 23 OF 29 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN DUPLICATE 3
TI Drosophila GABA-gated chloride channel: Modified [3H]EBOB binding site associated with Ala → Ser or Gly mutants of Rdl subunit.

L12 ANSWER 24 OF 29 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
TI BACILLUS-THURINGIENSIS PROTOXIN - LOCATION OF TOXIC BORDER AND REQUIREMENT OF NONTOXIC DOMAIN FOR HIGH-LEVEL IN-VIVO PRODUCTION OF ACTIVE TOXIN

L12 ANSWER 25 OF 29 MEDLINE on STN DUPLICATE 4
TI Molecular biology of **insecticide** resistance.

L12 ANSWER 26 OF 29 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
TI CLONING AND FUNCTIONAL EXPRESSION OF A DROSOPHILA GAMMA-AMINOBUTYRIC-ACID RECEPTOR

L12 ANSWER 27 OF 29 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
TI THE MOLECULAR AND POPULATION-GENETICS OF CYCLODIENE **INSECTICIDE** RESISTANCE

L12 ANSWER 28 OF 29 MEDLINE on STN
TI Cloning of a putative GABAA receptor from cyclodiene-resistant Drosophila: a case study in the use of **insecticide**-resistant mutants to isolate neuroreceptors.

L12 ANSWER 29 OF 29 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
TI Molecular dissection of cholinesterase domains responsible for carbamate toxicity.

=> d ibib abs L12 3-5, 13, 14, 17, 18, 20-22, 24, 25, 29

L12 ANSWER 3 OF 29 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2004:227205 BIOSIS
DOCUMENT NUMBER: PREV200400227221
TITLE: Introduction of Culex toxicity into Bacillus thuringiensis Cry4Ba by protein engineering.
AUTHOR(S): Abdullah, Mohd Amir F.; Alzate, Oscar; Mohammad, Marwan; McNall, Rebecca J.; Adang, Michael J.; Dean, Donald H. [Reprint Author]
CORPORATE SOURCE: Department of Biochemistry, Ohio State University, Columbus, OH, 43210-1292, USA
dean.10@osu.edu
SOURCE: Applied and Environmental Microbiology, (September 2003) Vol. 69, No. 9, pp. 5343-5353. print.
ISSN: 0099-2240 (ISSN print).
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 28 Apr 2004
Last Updated on STN: 28 Apr 2004
AB Bacillus thuringiensis mosquitocidal toxin Cry4Ba has no significant natural activity against Culex quinquefasciatus or Culex pipiens (50% lethal concentrations (LC50),>80,000 and >20,000 ng/ml, respectively). We introduced amino acid substitutions in three putative loops of **domain** II of Cry4Ba. The mutant proteins were tested on four different species of mosquitoes, Aedes aegypti, Anopheles quadrimaculatus, C. quinquefasciatus, and C. pipiens. Putative loop 1 and 2 exchanges eliminated activity towards A. aegypti and A. quadrimaculatus. **Mutations** in a putative loop 3 resulted in a final **increase** in **toxicity** of >700-fold and >285-fold against C. quinquefasciatus (LC50simeq114 ng/ml) and C. pipiens (LC50simeq37 ng/ml), respectively. The enhanced protein (mutein) has very little negative effect on the activity against Anopheles or Aedes. These results suggest that the introduction of short variable sequences of the loop regions from one **toxin** into another might provide a general rational design approach to **enhancing** B. thuringiensis Cry **toxins**.

L12 ANSWER 4 OF 29 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 2003:684817 SCISEARCH
THE GENUINE ARTICLE: 705PD
TITLE: Genotoxicity of methoxyphosphinyl **insecticide** in mammalian cells
AUTHOR: Wang T C (Reprint); Lin C M; Lo L W
CORPORATE SOURCE: Acad Sinica, Inst Zool, Taipei 115, Taiwan (Reprint)
COUNTRY OF AUTHOR: Taiwan
SOURCE: ZOOLOGICAL STUDIES, (JUL 2003) Vol. 42, No. 3, pp. 462-469
Publisher: ACAD SINICA INST ZOOLOGY, EDITORIAL OFFICE, TAIPEI 115, TAIWAN.
ISSN: 1021-5506.

DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 22
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The **genotoxicity** of 5 organophosphorus insecticides containing the methoxyphosphinyl **subunit** was assayed by examining the induction of sister-chromatid exchanges (SCEs), chromosome aberrations, and the hypoxanthine-guanine phosphoribosyl transferase (hgprt) gene **mutations** in CHO cells. Insecticides included acephate, dichlorvos, monocrotophos, methamidophos, and trichlorfon. They consistently induced significant SCEs, with the order of induction **potential** of acephate > trichlorfon > monocrotophos > methamidophos > dichlorvos. However, only 2 of them, dichlorvos and methamidophos, induced positive chromosome aberrations. Monocrotophos and acephate were questionable positive, while trichlorfon was negative for chromosome aberration induction. The order of chromosome aberration induction **potential** was dichlorvos > methamidophos > monocrotophos > acephate > trichlorfon. None of these 5 insecticides induced significant hgprt gene **mutations** compared to the concurrent negative control. The discrepancy between the results of the 2 cytogenetic endpoints, and the contradictory outcome between hgprt **mutation** and SCE are discussed, from which a possible mechanism of **insecticide genotoxicity** postulated.

L12 ANSWER 5 OF 29 EMBASE
on STN
ACCESSION NUMBER: 2002344365 EMBASE
TITLE: Effects of mutations of a glutamine residue in loop D of the α7 nicotinic acetylcholine receptor on agonist profiles for neonicotinoid insecticides and related ligands.
AUTHOR: Shimomura M.; Okuda H.; Matsuda K.; Komai K.; Akamatsu M.; Sattelle D.B.
CORPORATE SOURCE: K. Matsuda, Department of Agricultural Chemistry, Faculty of Agriculture, Kinki University, 3327-204 Nakamachi, Nara 631-8505, Japan. kmatsuda@nara.kindai.ac.jp
SOURCE: British Journal of Pharmacology, (2002) 137/2 (162-169).
Refs: 22
ISSN: 0007-1188 CODEN: BJPCBM
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB 1. Neonicotinoid insecticides are agonists of insect nicotinic acetylcholine receptors (AChRs) and show selective **toxicity** for insects over vertebrates. To elucidate the molecular basis of the selectivity, amino acid residues influencing neonicotinoid sensitivity were investigated by site-directed mutagenesis of the chicken α7 nicotinic AChR **subunit**, based on the crystal structure of an ACh binding protein (AChBP). 2. In the ligand binding site of AChBP, Q55 in loop D is close to Y164 in loop F that corresponds to G189 of the α7 nicotinic receptor. Since Q55 of AChBP is preserved as Q79 in the α7 nicotinic receptor and the G189D and G189E **mutations** have been found to reduce the neonicotinoid sensitivity, we investigated effects of Q79E, Q79K and Q79R **mutations** on the neonicotinoid sensitivity of the α7 receptor expressed in Xenopus laevis oocytes to evaluate contributions of the glutamine residue to nicotinic AChR-neonicotinoid interactions. 3. The Q79E **mutation** markedly reduced neonicotinoid sensitivity of the α7 nicotinic AChR whereas the Q79K and Q79R **mutations increased** sensitivity, suggesting electronic interactions of the neonicotinoids with the added residues. 4. By contrast, the Q79E **mutation** scarcely influenced responses of

the α7 nicotinic receptor to ACh, (-)-nicotine and desnitro-imidacloprid (DN-IMI), an imidacloprid derivative lacking the nitro group, whereas the Q79K and Q79R **mutations** reduced the sensitivity to these ligands. The results indicate that the glutamine residue of the α7 nicotinic receptor is likely to be located close to the nitro group of the insecticides in the nicotinic receptor-**insecticide** complex.

L12 ANSWER 13 OF 29 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1999:667139 CAPLUS
DOCUMENT NUMBER: 132:1223
TITLE: Identification of residues in domain III of Bacillus thuringiensis Cry1Ac toxin that affect binding and toxicity
AUTHOR(S): Lee, Mi Kyong; You, Taek H.; Gould, Fred L.; Dean, Donald H.
CORPORATE SOURCE: Department of Biochemistry, The Ohio State University, Columbus, OH, 43210, USA
SOURCE: Applied and Environmental Microbiology (1999), 65(10), 4513-4520
CODEN: AEMIDF; ISSN: 0099-2240
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Alanine substitution **mutations** in the Cry1Ac **domain** III region, from amino acid residues 503 to 525, were constructed to study the functional role of **domain** III in the toxicity and receptor binding of the protein to Lymantria dispar, Manduca sexta, and Heliothis virescens. Five sets of alanine block mutants were generated at the residues 503SS504, 506NNI508, 509QNR511, 522ST523, and 524ST525. Single alanine substitutions were made at the residues 509Q, 510N, 511R, and 513Y. All mutant proteins produced stable toxic fragments as judged by trypsin digestion, midgut enzyme digestion, and CD spectrum anal. The **mutations**, 503SS504-AA, 506NNI508-AAA, 522ST523-AA, 524ST525-AA, and 510N-A affected neither the protein's toxicity nor its binding to brush border membrane vesicles (BBMV) prepared from these insects. Toward L. dispar and M. sexta, the 509QNR511-AAA, 509Q-A, 511R-A, and 513Y-A mutant toxins showed 4- to 10-fold redns. in binding affinities to BBMV, with 2- to 3-fold redns. in toxicity. Toward H. virescens, the 509QNR511-AAA, 509Q-A, 511R-A, and 513Y-mutant toxins showed 8- to 22-fold redns. in binding affinities, but only for 509QNR511-AAA and 511R-A mutant toxins was toxicity reduced, by factors of approx. three to four. In the present study, **greater** loss in binding affinity relative to **toxicity** has been observed These data suggest that the residues 509Q, 511R, and 513Y in **domain** III might be only involved in initial binding to the receptor and that the initial binding step becomes rate limiting only when it is reduced more than fivefold.
REFERENCE COUNT: 51 THERE ARE 51 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 14 OF 29 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 1999457712 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10528405
TITLE: The pharmacological flexibility of the insect voltage gated sodium channel: toxicity of AaIT to knockdown resistant (kdr) flies.
AUTHOR: Zlotkin E; Devonshire A L; Warmke J W
CORPORATE SOURCE: Merck Research Laboratories, Rahway, NJ, USA.. zlotkin@vms.huji.ac.il
SOURCE: Insect biochemistry and molecular biology, (1999 Oct) 29 (10) 849-53.
Journal code: 9207282. ISSN: 0965-1748.
PUB. COUNTRY: ENGLAND: United Kingdom

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199912
ENTRY DATE: Entered STN: 20000113
Last Updated on STN: 20000113
Entered Medline: 19991202

AB AaIT is an insect selective neurotoxic polypeptide shown to affect insect neuronal sodium conductance by binding to excitable sodium channels. In the present study the paralytic potency of AaIT to wild type and various mutant strains of houseflies (Musca domestica) and fruitflies (Drosophila melanogaster) was examined and it has been shown that: On the basis of body weight when compared to published data on Sarcophaga falculata blowflies, the Musca and Drosophila flies reveal at least two orders of magnitude decreased susceptibility to the AaIT. When compared to wild type flies the **toxicity** of AaIT is **greatly** altered in knockdown resistant fly strains which are **mutated** in their para gene encoding the voltage gated sodium channel. Several strains, with genetically mapped para **mutations** conferring pyrethroid resistance, exhibited opposing response to AaIT. The para ts2 Drosophila strain, with a point of **mutation** in **domain** I of the para gene conferring a 6-fold resistance to deltamethrin also showed about 15-fold tolerance to AaIT. On the other hand the Musca kdr and super-kdr flies, with a single or a double point **mutation**, respectively in **domain** II of the para gene, are about 9- and 14-fold more susceptible to AaIT, respectively. The above data are interpreted in terms of the pharmacological diversity and flexibility ("allosteric coupling") of voltage gated sodium channels and their implications for the management of **pesticide** resistance are discussed.

L12 ANSWER 17 OF 29 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN DUPLICATE 2
ACCESSION NUMBER: 1999258140 EMBASE
TITLE: **Mutations** of loop 2 and loop 3 residues in **domain** II of Bacillus thuringiensis cry1c δ-endotoxin affect insecticidal specificity and initial binding to Spodoptera littoralis and Aedes aegypti midgut membranes.
AUTHOR: Abdul-Rauf M.; Ellar D.J.
CORPORATE SOURCE: D.J. Ellar, Department of Biochemistry, University of Cambridge, 80 Tennis Court Road, Cambridge CB2 1GA, United Kingdom
SOURCE: Current Microbiology, (1999) 39/2 (94-98).
Refs: 25
ISSN: 0343-8651 CODEN: CUMIDD
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Site-directed mutagenesis was used to examine the role of predicted loops 2 (374QPWP377) and 3 (436QRSGTPF442) in **domain** II of the Bacillus thuringiensis Cry1C δ **endotoxin** for insecticidal specificity and receptor binding. Q3764E, S438F, and G439A substitutions resulted in near or complete loss of **toxicity** toward both Spodoptera littoralis and Aedes aegypti. R437K, R437I, and G439V mutants exhibited significantly reduced **toxicity** to S. littoralis and A. aegypti, while **mutations** of T440, p441, and F442 showed only slight reductions in **toxicity** to both insects. Loop 2 **mutations** Q374N, P375A, W376Y, and P377A did not significantly affect S. littoralis **toxicity** but exhibited reduced activity to A. aegypti. In contrast, the loop 3 **mutations** Q436K, Q436E, and S438Y haft no effect on A. aegypti **toxicity**, but showed

significantly decreased S. littoralis activity. Heterologous competition binding assays with brush border membrane vesicles (BBMV) from both insects correlated well with the **toxicity** data with the exception of the R437 mutants, where steps other than initial receptor binding appear to be involved. Overall we conclude that, while loops 2 and 3 play an important role in binding and **toxicity** to both insects, loop 2 appears to play the **greater** role in A. aegypti activity, while loop 3 is **more** important for S. littoralis **toxicity**.

L12 ANSWER 18 OF 29 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
ACCESSION NUMBER: 1998-427565 [60] WPIDS
CROSS REFERENCE: 2000-182446 [16]
DOC. NO. CPI: C2003-171666
TITLE: Evolution of whole cells and organisms by recursive DNA sequence recombination in cells to evolve cells having acquired desired function, useful in methods for predicting the efficacy of a drug in treating viral or pathogenic infections.
DERWENT CLASS: B04 C06 D16
INVENTOR(S): DELCARDAYRE, S B; MINSHULL, J; NESS, J E; STEMMER, W P; TOBIN, M B; PATTEN, P; STEMMER, W P C; AFFHOLTER, J A; BASS, S; CASTLE, L A; COX, T; DEL CARDAYRE, S; HUISMAN, G; KREBBER, C M; SUBRAMANIAN, V; TOBIN, M; YUAN, L; ZHANG, Y; BASS, S H; CASTLE, L; DELCARDAYRE, S
PATENT ASSIGNEE(S): (MAXY-N) MAXYGEN INC
COUNTRY COUNT: 82
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9831837	A1	19980723	(200360)*	EN	124
RW: AT BE CH DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG ZW					
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM GW HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW					
AU 9859209	A	19980807	(199901)		
EP 1007732	A1	20000614	(200033)	EN	
R: AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE					
MX 9906637	A1	19991101	(200106)		
KR 2000070258	A	20001125	(200131)		
US 6251674	B1	20010626	(200138)		
JP 2001508662	W	20010703	(200142)		122
US 6287862	B1	20010911	(200154)		
US 6326204	B1	20011204	(200203)		
US 6335198	B1	20020101	(200207)		
AU 743305	B	20020124	(200221)		
US 6352859	B1	20020305	(200224)		
AU 2002010224	A	20020314	(200227)#		
US 6379964	B1	20020430	(200235)		
US 6528311	B1	20030304	(200320)		
US 2003148309	A1	20030807	(200358)		
US 6716631	B1	20040406	(200425)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9831837	A1	WO 1998-US852	19980116
AU 9859209	A	AU 1998-59209	19980116
EP 1007732	A1	EP 1998-902586	19980116

			WO 1998-US852	19980116
MX 9906637	A1		MX 1999-6637	19990716
KR 2000070258	A		WO 1998-US852	19980116
			KR 1999-706486	19990716
US 6251674	B1	Provisional	US 1997-35054P	19970117
		Div ex	WO 1998-US852	19980116
		Div ex	US 1998-116188	19980715
			US 2000-499505	20000207
JP 2001508662	W		JP 1998-534558	19980116
			WO 1998-US852	19980116
US 6287862	B1	Provisional	US 1997-35054P	19970117
		CIP of	WO 1998-US852	19980116
		Div ex	US 1998-116188	19980715
			US 2000-626410	20000726
US 6326204	B1	Provisional	US 1997-35054P	19970117
		CIP of	WO 1998-US852	19980116
			US 1998-116188	19980715
US 6335198	B1	Provisional	US 1997-35054P	19970117
		CIP of	WO 1998-US852	19980116
		Div ex	US 1998-116188	19980715
			US 2000-626047	20000726
AU 743305	B		AU 1998-59209	19980116
US 6352859	B1	Provisional	US 1997-35054P	19970117
		Div ex	WO 1998-US852	19980116
		Div ex	US 1998-116188	19980715
			US 2000-626343	20000726
AU 2002010224	A	Div ex	AU 1998-59209	19980116
			AU 2002-10224	20020117
US 6379964	B1	Provisional	US 1997-35054P	19970117
		CIP of	WO 1998-US852	19980116
		CIP of	US 1998-116188	19980715
			US 1999-354922	19990715
US 6528311	B1	Provisional	US 1997-35054P	19970117
		CIP of	WO 1998-US852	19980116
		Cont of	US 1998-116188	19980715
		CIP of	US 2000-516051	20000301
			US 2000-516051	20000301
US 2003148309	A1	Provisional	US 1997-35054P	19970117
		CIP of	WO 1998-US852	19980116
		CIP of	US 1998-116188	19980715
		Cont of	US 1999-354922	19990715
		Cont of	US 2000-718262	20001121
			US 2002-194686	20020711
US 6716631	B1	Provisional	US 1997-35054P	19970117
		CIP of	WO 1998-US852	19980116
		Div ex	US 1998-116188	19980715
			US 2000-516695	20000301

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9859209	A	Based on	WO 9831837
EP 1007732	A1	Based on	WO 9831837
KR 2000070258	A	Based on	WO 9831837
JP 2001508662	W	Based on	WO 9831837
AU 743305	B	Previous Publ.	AU 9859209
		Based on	WO 9831837
AU 2002010224	A	Div ex	AU 743305
US 6528311	B1	Cont of	US 6326204
US 2003148309	A1	CIP of	US 6326204
		Cont of	US 6379964
US 6716631	B1	Div ex	US 6326204

PRIORITY APPLN. INFO: US 1997-35054P 19970117; US
1998-116188 19980715; US
2000-499505 20000207; US
2000-626410 20000726; US
2000-626047 20000726; US
2000-626343 20000726; AU
2002-10224 20020117; US
1999-354922 19990715; US
2000-516051 20000301; US
2000-718262 20001121; US
2002-194686 20020711; US
2000-516695 20000301

AN 1998-427565 [60] WPIDS

CR 2000-182446 [16]

AB WO 9831837 A UPAB: 20040418

NOVELTY - Employing (M1) iterative cycles of recombination and selection/screening for evolution of whole cells and organisms towards acquisition of desired functions and properties, is new.

DETAILED DESCRIPTION - A method (M1) employing iterative cycles of recombination and selection/screening for evolution of whole cells and organisms towards acquisition of desired functions and properties, comprising:

(a) introducing a library of DNA fragments into cells where at least one of the fragments undergoes recombination with a segment in the genome or an episome of the cells to produce modified cells;

(b) screening the modified cells for modified cells that have evolved toward the desired function;

(c) recombining DNA from the modified cells that have evolved toward the desired function with a further library of DNA fragments at least one of which undergoes recombination with a segment in the genome or the episome of the modified cells to produce further modified cells;

(d) screening the further modified cells for cells that have further evolved toward acquisition of the desired function; and

(e) repeating (c) and (d) as required until the further modified cells have acquired the desired function.

INDEPENDENT CLAIMS are also included for:

(1) enhancing (M2) tissue-specific expression of a protein in a transgenic animal, comprising:

(a) recombining at least first and second forms of a gene encoding a protein, the forms differing from each other in at least two nucleotides, to produce a library of chimeric genes;

(b) screening the library to identify at least one chimeric gene, which as a component of a transgene, confers enhanced expression of the protein in cells from the tissue relative to a transgene containing the wildtype form of the gene;

(c) recombining the at least one chimeric gene with a further form of the gene, the same or different from the first and second forms, to produce a further library of chimeric genes;

(d) screening the further library for at least one further chimeric gene that as a component of a transgene confers enhanced expression of the protein in cells from the tissue relative to a transgene comprising the chimeric gene in the previous screening step;

(e) repeating (c) and (d), as necessary, until the further chimeric gene confers a desired level of expression in cells from the tissue;

(2) performing (M3) in vivo recombination, comprising:

(a) providing a cell incapable of expressing a cell septation gene;

(b) introducing at least first and second segments from at least one gene into a cell, the segments differing from each other in at least two nucleotides, where the segments recombine to produce a library of chimeric genes;

(c) selecting a chimeric gene from the library having an acquired function;

(3) predicting (M4) efficacy of a drug in treating a viral infection (e.g. HIV infection) or an infection by a pathogenic microorganism;

(4) a recA protein selected from clone 2, clone 4, clone 5, clone 6 and clone 13 as defined in the specification; and

(5) evolving a recA protein to increase recombinogenic activity, comprising:

(a) shuffling a population of nucleic acid segments encoding variants of recA including a nucleic acid segment selected from clone 2, clone 4, clone 5, clone 6 and clone 13 as defined in the specification, to produce recombinant segments; and

(b) screening or selecting a recombinant segment with increased recombinogenic activity relative to the nucleic acid segment selected from the group.

USE - The methods can be used to evolve the genomes of cells and organisms to acquire new and improved properties. They can be used to improve the properties of bacterial, archaebacteria, eukaryotic or plant cells. They can also be used for predicting the efficacy of a drug in treating viral or pathogenic infections.

Dwg.0/13

L12 ANSWER 20 OF 29 EMBASE
on STN

ACCESSION NUMBER: 96378850 EMBASE
DOCUMENT NUMBER: 1996378850
TITLE: Protein engineering of Bacillus thuringiensis δ-**endotoxin**: **Mutations** at **domain** II of CryIAb **enhance** receptor affinity and **toxicity** toward gypsy moth larvae.
AUTHOR: Rajamohan F.; Alzate O.; Cotrill J.A.; Curtiss A.; Dean D.H.
CORPORATE SOURCE: Biophysics Program, Ohio State University,Columbus, OH 43210-1292, United States
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1996) 93/25 (14338-14343). ISSN: 0027-8424 CODEN: PNASA6
COUNTRY: United States
DOCUMENT TYPE: Journal; Conference Article
FILE SEGMENT: 004 Microbiology
022 Human Genetics
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Substitutions or deletions of domain II loop residues of Bacillus thuringiensis δ- **endotoxin** CryIAb were constructed using site-directed mutagenesis techniques to investigate their functional roles in receptor binding and **toxicity** toward gypsy moth (Lymantria dispar). Substitution of loop 2 residue N372 with Ala or Gly (N372A, N372G) **increased** the **toxicity** against gypsy moth larvae 8-fold and **enhanced** binding affinity to gypsy moth midgut brush border membrane vesicles (BBMV) .simeq.4-fold. Deletion of N372 (D3), however, substantially reduced **toxicity** (>21 times) as well as binding affinity, suggesting that residue N372 is involved in receptor binding. Interestingly, a triple mutant, DF-1 (N372A, A282G and L283S), has a 36-fold **increase** in **toxicity** to gypsy moth neonates compared with wild-type **toxin**. The **enhanced** activity of DF-1 was correlated with higher binding affinity (18-fold) and binding site concentrations. Dissociation binding assays suggested that the off-rate of the BBMV-bound mutant **toxins** was similar to that of the wild type. However, DF-1 **toxin** bound 4 times **more** than the wild- type and N372A **toxins**, and it was directly correlated with binding affinity and potency. Protein blots of gypsy moth BBMV probed with labeled N372A, DF- 1, and CryIAb **toxins** recognized a common 210-kDa protein, indicating that the **increased** activity of the mutants was not caused by binding to

additional receptor(s). The improved binding affinity of N372A and DF-1 suggest that a shorter side chain at these loops may fit the **toxin** **more** efficiently to the binding pockets. These results offer an excellent model system for engineering δ- **endotoxins** with higher potency and wider spectra of target pests by improving receptor binding interactions.

L12 ANSWER 21 OF 29 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 96226400 EMBASE
DOCUMENT NUMBER: 1996226400
TITLE: Mutagenesis of three surface-exposed loops of a Bacillus thuringiensis insecticidal toxin reveals residues important for toxicity, receptor recognition and possibly membrane insertion.
AUTHOR: Smedley D.P.; Ellar D.J.
CORPORATE SOURCE: Department of Biochemistry, University of Cambridge, Tennis Court Road,Cambridge CB2 1QW, United Kingdom
SOURCE: Microbiology, (1996) 142/7 (1617-1624).
ISSN: 1350-0872 CODEN: MROBEO
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Information on the molecular determinants of receptor recognition, membrane insertion and **toxin** pore-formation was sought by making 42 single and multiple substitutions of residues 312-314 (GYY), 367-370 (YRRP) and 438-441 (SGFS) in the Bacillus thuringiensis insecticidal CrylAc δ- **endotoxin** by site-directed mutagenesis. These three regions correspond to three putative surface-exposed loops (loops 1, 2 and 3, respectively) in **domain** II of the δ- **endotoxin,** forming the molecular apex of the structure. All except mutants GFY (loop 1), YKRA, SRRA, YRKA (loop 2) and TGFS (loop 3) expressed δ- **endotoxin** protein at wild-type levels which was stable upon activation by Pieris brassicae gut extract or trypsin. **Toxicity** assays for all the fully stable mutants using Manduca sexta larvae showed that G312, Y367, R368, R369, S438 and G439 are important for activity. Wild-type **toxin** was then labelled in vivo with [35S]methionine and heterologous competition binding assays were carried out for all the mutants using brush border membrane vesicles prepared from Manduca sexta midgut. Most and least conservative **mutations** of G439 and least conservative substitutions of Y367, R368 and R369 reduced the ability of the **toxin** to bind competitively. The most conservative **mutation,** S441T, gave significantly **increased** binding. These results suggested that these four residues play a role in the initial receptor binding step in the **toxin** mechanism. As no significant effect on binding affinity was observed in relatively non-**toxic** mutants in which residues G312 and S438 were **mutated,** we suggest that these residues are involved in the subsequent steps of membrane insertion and pore-formation.

L12 ANSWER 22 OF 29 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1996:118068 BIOSIS
DOCUMENT NUMBER: PREV199698690203
TITLE: Functional significance of loops in the receptor binding domain of Bacillus thuringiensis CryIIIA delta-endotoxin.
AUTHOR(S): Wu, Sheng-Jiun; Dean, Donald H. [Reprint author]
CORPORATE SOURCE: Dep. Biochem., Ohio State Univ., Columbus, OH 43210, USA
SOURCE: Journal of Molecular Biology, (1996) Vol. 255, No. 4, pp. 628-640.
CODEN: JMOBAK. ISSN: 0022-2836.

DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 27 Mar 1996
Last Updated on STN: 27 Mar 1996

AB Analysis of the three surface loops in **domain** II of Bacillus thuringiensis CryIIIA delta-endotoxin has been carried out to assess their role in receptor binding and toxicity. Site-directed mutagenesis was used to convert loop residues to alanine and the mutant proteins were analyzed for structural stability toxicity to beetle larvae (Tenebrio molitor), binding to receptors on T. molitor brush border membrane vesicles (Tm-BBMV) and insertion into BBMV, as measured by irreversible membrane receptor binding. This study demonstrates the functional significance of loops for binding and insertion. Alanine replacements in loop I resulted in disruption of receptor binding or structural instability The double **mutation**, Y-350A,Y-351A, could be suppressed by replacing a nearby R-345 with alanine, and the resultant mutant protein also showed reduced receptor binding. Substitution of N-353 and D-354 in loop I with alanine residues caused the loss of binding ability and **toxicity** A loop II double mutant, P-412A,S-413A, had no effect on binding or **toxicity** A block **mutation** of loop III residues to alanine had the effect of reducing receptor binding while concomitantly **increasing toxicity** by 2.4-fold. We compared this up-mutant to wild-type toxin in each step of physiological processing of protoxin: solubility proteolytic activation, and insertion into the Tm-BBMV. The loop III block mutant showed **increased** membrane insertion, but was similar to wild-type **toxin** in other parameters. These results reveal that loop I and loop III in **domain** II of CryIIIA delta-endotoxin are involved in receptor binding. In addition, the direct correlation between toxicity and irreversible binding of the loop III block mutant (despite the indirect relationship to reversible binding) suggests that loop III may play a role in membrane insertion.

L12 ANSWER 24 OF 29 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 95:221032 SCISEARCH
THE GENUINE ARTICLE: QN079
TITLE: BACILLUS-THURINGIENSIS PROTOXIN - LOCATION OF TOXIC BORDER AND REQUIREMENT OF NONTOXIC DOMAIN FOR HIGH-LEVEL IN-VIVO PRODUCTION OF ACTIVE TOXIN
AUTHOR: WABIKO H (Reprint); YASUDA E
CORPORATE SOURCE: AKITA PREFECTURAL COLL AGR, INST BIOTECHNOL, 2-2 MINAMI, OHGATA, AKITA 01004, JAPAN (Reprint)
COUNTRY OF AUTHOR: JAPAN
SOURCE: MICROBIOLOGY-UK, (MAR 1995) Vol. 141, Part 3, pp. 629-639. ISSN: 1350-0872.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 40

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Insecticidal crystal proteins, or **protoxins**, of Bacillus thuringiensis are composed of two domains, an amino-terminal half essential for **toxicity**, and a carboxy-terminal half with an as yet unassigned function. To define the boundary of the two domains, sequential termination codons were introduced from the 3'-end of the DNA sequence encoding the **toxic domain** of the 1155-residue cry1A(b) gene product. The **mutated** and the intact genes were placed under the control of the Escherichia coil inducible promoter PrecA, and **toxicity** of the cell extracts was determined using silkworm larvae. Under non-induced conditions, in which the gene products accumulated to a limited degree, **mutations** encoding 606 amino acid residues or **more** were **toxic**, whereas those encoding 605 residues or less were non-**toxic**. Comparison of the

toxicities and the levels of the **toxic** proteins suggested that the mutant proteins had comparable activity to that of the intact **protoxin**. Furthermore, the non-**toxic** protein seemed to be unstable in the extracts. To investigate the roles of the non-**toxic domain**, the mutant proteins were overproduced in both E. coil and B. thuringiensis. The intact and the **mutated** genes carrying natural promoters were introduced into acrystalliferous B. thuringiensis. Upon induction of PrecA in E. coil, and upon sporulation in B. thuringiensis, there was a large accumulation of gene products which formed inclusion bodies. The inclusion bodies of the intact **protoxin** were active, whereas those of the mutant proteins were inactive. Inclusion bodies of the intact protein could be solubilized in alkali, whereas the mutant inclusion bodies were insoluble. Since solubilization under alkaline conditions in the insect midgut is considered to be the first step of **toxic** action, the non-**toxic domain** is required to direct the synthesis of inclusion bodies as an active soluble form.

L12 ANSWER 25 OF 29 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 96170075 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8597150
TITLE: Molecular biology of **insecticide** resistance.
AUTHOR: Feyereisen R
CORPORATE SOURCE: Department of Entomology, University of Arizona, Tucson 85721, USA.
SOURCE: Toxicology letters, (1995 Dec) 82-83 83-90. Ref: 18
Journal code: 7709027. ISSN: 0378-4274.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199604
ENTRY DATE: Entered STN: 19960424
Last Updated on STN: 19960424
Entered Medline: 19960415

AB The widespread use of insecticides has amounted to a large scale 'experiment' in natural selection of insects by chemicals of toxicological importance to humans. Specific examples in which the molecular basis of **insecticide** resistance has been studied in detail are presented here. The biochemical/physiological mechanisms of resistance can be categorized as target site insensitivity, **increased** metabolic **detoxification** and sequestration or lowered availability of the **toxicant**. These are achieved at the molecular level by: point **mutations** in the ion channel portion of a GABA receptor **subunit** (cyclodiene insecticides); point **mutations** in the vicinity of the acetylcholinesterase (AChE) active site (organophosphorus and carbamate **insecticide** resistance); amplification of esterase genes (organophosphorus and carbamate insecticides); **mutations** linked genetically to a sodium channel gene (DDT and pyrethroid insecticides); and yet uncharacterized **mutations** leading to the up-regulation of detoxification enzymes, such as cytochrome P450 and glutathione S-transferases (many classes of insecticides). In several cases, the selection of a precisely homologous **mutation** has been observed in different insect species.

L12 ANSWER 29 OF 29 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 93205395 EMBASE
DOCUMENT NUMBER: 1993205395
TITLE: Molecular dissection of cholinesterase domains responsible for carbamate toxicity.

AUTHOR: Loewenstein Y.; Denarie M.; Zakut H.; Soreq H.
CORPORATE SOURCE: Department of Biological Chemistry, Life Sciences Institute, Hebrew University,Jerusalem 91904, Israel
SOURCE: Chemico-Biological Interactions, (1993) 87/1-3 (209-216). ISSN: 0009-2797 CODEN: CBINA8
COUNTRY: Ireland
DOCUMENT TYPE: Journal; Conference Article
FILE SEGMENT: 029 Clinical Biochemistry
052 Toxicology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Carbamate compounds marked for their cholinesterase (ChE) inhibition are widely used as therapeutics and as insecticides. Groups of closely related carbamate molecules provide an important tool in the understanding of the domains responsible for binding these ligands to ChEs. Comparative inhibition profiles were derived for five N-methyl carbamates, mostly carbofuran derivatives, differing in length and branching of their hydrocarbonic chain towards human erythrocyte acetylcholinesterase (H.AChE), human serum butyrylcholinesterase (H.BChE) in its normal form or in a mutant form containing the point **mutation** Asp70 → Gly, and Drosophila nervous system ChE. Carbofuran was **more toxic** to all three ChEs than any of the other derivatives, with IC50 values which differed by **more** than 1000-fold. Drosophila ChE appeared to be most sensitive to all of the examined crhbamates, and H.AChE was consistently **more** sensitive than H.BChE. Morever, inhibition efficiency for H.BChE decreased **more** effectively than it did for H.AChE with **increased** length and complexity of the side chain, indicating less flexible carbamate binding site in BChE as compared with AChE. The Asp70 → Gly **mutation** had no apparent effect on H.BChE inhibition by N-methyl carbamates, suggesting that the Asp70 **domain** localized near the rim of the active site groove is not important in carbamate binding. Comparison of the carbamate IC50 values with published LD50 values demonstrated correlation between the in vivo **toxicity** and inhibition of BChE by carbamates, suggesting a biological in addition to scavenging importance for BChE in mammals. Pinpointing different domains characteristic of carbamate binding in each member of the ChE family can thus shed light on the variable **toxicity** of these inhibitors to insects and mammals, predict the **toxicity** of yet untested inhibitor molecules and help in designing novel and improved ChE inhibitors.

=> FIL STNGUIDE

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	236.45	236.66

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
CA SUBSCRIBER PRICE	-0.74	-0.74

FILE 'STNGUIDE' ENTERED AT 20:24:57 ON 28 JUL 2004

FILE CONTAINS CURRENT INFORMATION.
LAST RELOADED: Jul 23, 2004 (20040723/UP).

=> file medline biosis embase scisearch caplus wpids

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	0.30	236.96

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DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)        SINCE FILE      TOTAL
                                                      ENTRY    SESSION
CA SUBSCRIBER PRICE                                    0.00      -0.74

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FILE 'BIOSIS' ENTERED AT 20:27:49 ON 28 JUL 2004
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FILE 'WPIDS' ENTERED AT 20:27:49 ON 28 JUL 2004
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=> s Shiga (w) toxin
L13         5982 SHIGA (W) TOXIN

=> d his

     (FILE 'HOME' ENTERED AT 19:54:54 ON 28 JUL 2004)

     FILE 'MEDLINE, BIOSIS, EMBASE, SCISEARCH, CAPLUS, WPIDS' ENTERED AT
     19:55:20 ON 28 JUL 2004
L1             2 S (MUTAT (S) (SUBUNIT OR DOMAIN)) (P) ((INCREAS OR MORE OR GREA
L2             4 S (MUTAT (S) (SUBUNIT OR DOMAIN)) (P) ((INCREAS? OR MORE OR GRE
L3             3 DUP REM L2 (1 DUPLICATE REMOVED)
L4             1 S L3 NOT L1
L5             0 S (INSECTICIDE OR HERBICIDE OR PESTICIDE) AND (MUTAT (S) (SUBUN
L6             0 S (INSECTICIDE OR HERBICIDE OR PESTICIDE) AND (MUTAT (P) (SUBUN
L7        407169 S INSECTICIDE OR HERBICIDE OR PESTICIDE
L8            72 S  (MUTAT (P) (SUBUNIT OR DOMAIN))
L9           389 S (INSECTICIDE OR HERBICIDE OR PESTICIDE) AND (MUTAT? (P) (SUBU
L10       920196 S (INCREAS? OR MORE OR GREAT? OR ENHANC? OR POTENTIA?) (S) (?TO
L11           38 S L10 AND L9
L12           29 DUP REM L11 (9 DUPLICATES REMOVED)

     FILE 'STNGUIDE' ENTERED AT 20:24:57 ON 28 JUL 2004

     FILE 'MEDLINE, BIOSIS, EMBASE, SCISEARCH, CAPLUS, WPIDS' ENTERED AT
     20:27:49 ON 28 JUL 2004
L13         5982 S SHIGA (W) TOXIN

=> s  (mutat? (p) (subunit or domain))
L14       165943 (MUTAT? (P) (SUBUNIT OR DOMAIN))

=> L13 and L14 and L10
L13 IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> s L13 and L14 and L10
L15           18 L13 AND L14 AND L10
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=> dup rem
ENTER L# LIST OR (END):l15
PROCESSING COMPLETED FOR L15
L16             8 DUP REM L15 (10 DUPLICATES REMOVED)

=> t ti l16 1-8

L16  ANSWER 1 OF 8  EMBASE  COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED.
     on STN                                              DUPLICATE 1
TI   Effects of HIV-1 Nef on retrograde transport from the plasma membrane to
     the endoplasmic reticulum.

L16  ANSWER 2 OF 8  SCISEARCH  COPYRIGHT 2004 THOMSON ISI on STN
TI   Exogenous peptides delivered by ricin require processing by signal
     peptidase for transporter associated with antigen processing-independent
     MHC class I-restricted presentation

L16  ANSWER 3 OF 8  WPIDS  COPYRIGHT 2004 THOMSON DERWENT on STN
TI   Recombinant AB5B subunit protein comprising a mutation
     that alters the number residues available for chemical modification,
     useful for covalently linking to an immunogen or vaccine which can be used
     for treating autoimmune diseases.

L16  ANSWER 4 OF 8  WPIDS  COPYRIGHT 2004 THOMSON DERWENT on STN
TI   Protein transduction system for treating cancer and pathogenic infections
     has a fusion protein comprising a protein transduction domain covalently
     linked to a cytotoxic domain.

L16  ANSWER 5 OF 8  WPIDS  COPYRIGHT 2004 THOMSON DERWENT on STN
TI   Expression cassette used as live vector vaccine comprises nucleotide
     sequence encoding origin of replication and plasmid maintenance system
     which includes a post-segregational killing and a partitioning function.

L16  ANSWER 6 OF 8     MEDLINE on STN                   DUPLICATE 2
TI   Disruption of an internal membrane-spanning region in Shiga
     toxin 1 reduces cytotoxicity.

L16  ANSWER 7 OF 8     MEDLINE on STN                   DUPLICATE 3
TI   Role of the disulfide bond in Shiga toxin A-chain for
     toxin entry into cells.

L16  ANSWER 8 OF 8     MEDLINE on STN                   DUPLICATE 4
TI   Entry of Shiga toxin into cells.


=> d ibib abs L16 1-8

L16  ANSWER 1 OF 8  EMBASE  COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED.
     on STN                                              DUPLICATE 1
ACCESSION NUMBER:   2003202412  EMBASE
TITLE:              Effects of HIV-1 Nef on retrograde transport from the
                    plasma membrane to the endoplasmic reticulum.
AUTHOR:             Johannes L.; Pezo V.; Mallard F.; Tenza D.; Wiltz A.;
                    Saint-Pol A.; Helft J.; Antony C.; Benaroch P.
CORPORATE SOURCE:   L. Johannes, CNRS UMR144, Institut Curie, 26 rue d'Ulm,
                    F-75248 Paris Cedex 05, France. Ludger.Johannes@curie.fr
SOURCE:             Traffic, (1 May 2003) 4/5 (323-332).
                    Refs: 42
                    ISSN: 1398-9219  CODEN: TRAFFA
COUNTRY:            Denmark
DOCUMENT TYPE:      Journal; Article
FILE SEGMENT:       004     Microbiology
```

LANGUAGE: English
SUMMARY LANGUAGE: English
AB HIV-1 Nef protein down-regulates several important immunoreceptors through interactions with components of the intracellular sorting machinery. Nef expression is also known to induce modifications of the endocytic pathway. Here, we analyzed the effects of Nef on retrograde transport, from the plasma membrane to the endoplasmic reticulum using **Shiga toxin** B-**subunit** (STxB). Nef expression inhibited access of STxB to the endoplasmic reticulum, but did not modify the surface expression level of STxB receptor, Gb(3), nor its internalization rate as measured with a newly developed assay. **Mutation** of the myristoylation site or of a di-leucine motif of Nef involved in the interaction with the clathrin adaptor complexes AP1 and AP2 abolished the inhibition of retrograde transport. In contrast, **mutations** of Nef motifs known to interact with PACS-1, βCOP or a **subunit** of the v-ATPase did not modify the inhibitory activity of Nef on retrograde transport. Ultrastructural analysis revealed that Nef was present in clusters located on endosomal or Golgi membranes together with internalized STxB. Furthermore, in strongly Nef-expressing cells, STxB accumulated in endosomal structures that labeled with AP1. Our observations show that Nef perturbs retrograde transport between the early endosome and the endoplasmic reticulum. The **potential** transport steps targeted by Nef are discussed.

L16 ANSWER 2 OF 8 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 2002:533185 SCISEARCH
THE GENUINE ARTICLE: 565GA
TITLE: Exogenous peptides delivered by ricin require processing by signal peptidase for transporter associated with antigen processing-independent MHC class I-restricted presentation
AUTHOR: Smith D C; Gallimore A; Jones E; Roberts B; Lord J M; Deeks E; Cerundolo V; Roberts L M (Reprint)
CORPORATE SOURCE: Univ Warwick, Dept Biol Sci, Coventry CV4 7AL, W Midlands, England (Reprint); John Radcliffe Hosp, Mol Immunol Grp, Nuffield Dept Med, Oxford OX3 9DU, England
COUNTRY OF AUTHOR: England
SOURCE: JOURNAL OF IMMUNOLOGY, (1 JUL 2002) Vol. 169, No. 1, pp. 99-107.
Publisher: AMER ASSOC IMMUNOLOGISTS, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814 USA.
ISSN: 0022-1767.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 58
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB In this study we demonstrate that a disarmed version of the **cytotoxin** ricin can deliver exogenous CD8(+) T cell epitopes into the MHC class I-restricted pathway by a TAP-independent, signal peptidase-dependent pathway. Defined viral peptide epitopes genetically fused to the N terminus of an attenuated ricin A **subunit** (kTA) that was reassociated with its partner B **subunit** were able to reach the early secretory pathway of sensitive cells, including TAP-deficient cells. Successful processing and presentation by MHC class I proteins was not dependent on proteasome Activity or on recycling of MHC class I proteins, but rather on a functional secretory pathway. Our results demonstrated a role for signal peptidase in the generation of peptide epitopes associated at the amino terminus of RTA. We showed, first, that **potential** signal peptide cleavage sites located toward the N terminus of RTA can be posttranslationally cleaved by signal peptidase and, second, that **mutation** of one of these sites led to a loss of peptide presentation. These results identify a novel MHC class I presentation pathway that exploits the ability of **toxins**

to reach the lumen of the endoplasmic reticulum by retrograde transport, and suggest a role for endoplasmic reticulum signal peptidase in the processing and presentation of MHC class I peptides. Because. TAP-negative cells can be sensitized for CTL killing following retrograde transport of **toxin**-linked peptides, application of these results has direct implications for the development of novel vaccination strategies.

L16 ANSWER 3 OF 8 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
ACCESSION NUMBER: 2001-281974 [29] WPIDS
DOC. NO. CPI: C2001-085907
TITLE: Recombinant AB5B **subunit** protein comprising a **mutation** that alters the number residues available for chemical modification, useful for covalently linking to an immunogen or vaccine which can be used for treating autoimmune diseases.
DERWENT CLASS: B04 D16
INVENTOR(S): EWALT, K L; HAAPARANTA, T; HANDLEY, H H
PATENT ASSIGNEE(S): (ACTI-N) ACTIVE BIOTECH AB; (SBLV-N) SBL VACCIN AB
COUNTRY COUNT: 95
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2001027144	A2	20010419	(200129)*	EN	78
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TZ UG ZW					
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW					
AU 2000078659	A	20010423	(200147)		
EP 1222202	A2	20020717	(200254)	EN	
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI					
JP 2003511061	W	20030325	(200330)		119
NZ 518342	A	20040430	(200431)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2001027144	A2	WO 2000-US27607	20001005
AU 2000078659	A	AU 2000-78659	20001005
EP 1222202	A2	EP 2000-968795	20001005
		WO 2000-US27607	20001005
JP 2003511061	W	WO 2000-US27607	20001005
		JP 2001-530362	20001005
NZ 518342	A	NZ 2000-518342	20001005
		WO 2000-US27607	20001005

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 2000078659	A	Based on	WO 2001027144
EP 1222202	A2	Based on	WO 2001027144
JP 2003511061	W	Based on	WO 2001027144
NZ 518342	A	Based on	WO 2001027144

PRIORITY APPLN. INFO: US 1999-158561P 19991008
AN 2001-281974 [29] WPIDS
AB WO 200127144 A UPAB: 20040608
NOVELTY - A recombinant AB5B **subunit** protein (P1) comprising at

least one **mutation**, where the **mutation** alters the number of amino acid residues available for chemical modification as compared to a wild type AB5B **subunit** protein, and where the recombinant protein retains an effective target ligand binding affinity, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a method of making a recombinant AB5B **subunit** gene encoding the recombinant P1 protein, comprising providing an AB5B **subunit** gene encoding P1 protein, selecting codons encoding amino acid residues involved in covalent modification of the P1 protein, and **mutating** the codons such that the resulting amino acids are either incapable of covalent modification or possess enhanced modification capabilities;

(2) a method for producing the recombinant P1 protein, comprising obtaining a gene encoding the recombinant P1, adding to the gene a promoter, therefore producing an expression cassette, introducing the expression cassette into a suitable host cell, and cultivating the host cell under conditions where the expression cassette is translated into protein;

(3) a gene construct (N1) for producing the recombinant P1, comprising a promoter and a DNA sequence which encodes the recombinant P1, operably linked in the proper reading frame;

(4) a method for producing the recombinant P1, comprising expressing N1 in a suitable host cell and recovering recombinant P1;

(5) a method (M1) of generating an immune response to a recombinant P1, comprising providing P1, covalently modifying the protein with a dimeric cross-linking reagent with a first and a second functional group, where the first functional group is in chemical association with the recombinant P1, covalently modifying the second functional group with a compound, and administering the modified protein to a host until the immune response is generated; and

(6) an expression vector comprising a promoter and a gene encoding for P1, where P1 is selected from cholera toxin B protein (CTB) (preferred), E. coli heat toxin B protein (LTB), LT type IIa B protein, LT type IIb B protein, **Shiga toxin** B protein, Shiga like toxin B protein, or pertussis toxin B protein.

ACTIVITY - Immunosuppressive; antiarthritic; antirheumatic; antidiabetic; neuroprotective.

No biological data given.

MECHANISM OF ACTION - Vaccine.

No biological data given.

USE - A recombinant AB5B **subunit** protein such as CTB can be specifically covalently linked at lysines or cysteines to an immunogen or vaccine. Recombinant mutant CTB (rCTB) can be used in the treatment of autoimmune diseases e.g. rheumatoid arthritis, encephalomyelitis (or other neuron demyelinating diseases) and diabetes.

The rCTB or other B subunits of the invention can also be used to induce tolerance to infection, e.g. parasitic infection.

Dwg.0/3

L16 ANSWER 4 OF 8 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN

ACCESSION NUMBER: 2000-431269 [37] WPIDS

DOC. NO. CPI: C2000-131046

TITLE: Protein transduction system for treating cancer and pathogenic infections has a fusion protein comprising a protein transduction domain covalently linked to a cytotoxic domain.

DERWENT CLASS: B04 D16

INVENTOR(S): DOWDY, S F

PATENT ASSIGNEE(S): (UNIW) UNIV WASHINGTON

COUNTRY COUNT: 87

PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2000034308	A2	20000615	(200037)*	EN	127
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW					
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG UZ VN YU ZA ZW					
AU 2000021728	A	20000626	(200045)		
EP 1137664	A2	20011004	(200158)	EN	
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI					
JP 2002531113	W	20020924	(200278)		173

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2000034308	A2	WO 1999-US29289	19991210
AU 2000021728	A	AU 2000-21728	19991210
EP 1137664	A2	EP 1999-966101	19991210
		WO 1999-US29289	19991210
JP 2002531113	W	WO 1999-US29289	19991210
		JP 2000-586751	19991210

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 2000021728	A	Based on	WO 2000034308
EP 1137664	A2	Based on	WO 2000034308
JP 2002531113	W	Based on	WO 2000034308

PRIORITY APPLN. INFO: US 1998-111701P 19981210

AN 2000-431269 [37] WPIDS

AB WO 200034308 A UPAB: 20000807

NOVELTY - Protein transduction system (I) comprising a fusion protein (F) has a covalently linked protein transduction domain (D1) and cytotoxic domain (D2).

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a substantially pure (F);

(2) a nucleic acid segment (II) encoding (F);

(3) a DNA vector comprising (II);

(4) screening for a candidate compound to inhibit a pathogens-specific protease comprising transducing (F) into a cell population, expressing the protease by infecting with the pathogen, contacting the protease with (F) to produce a cytotoxin and modulating the protease;

(5) a kit comprising (I);

(6) introducing (F) into a cell by isolating (F) from a host cell, misfolding (F) and transducing it into the cell; and

(7) a protein transduction domain represented by or comprising at least a peptide of the following formulae:

B1-X1-X2-X3-B2-X4-X5-B3 or B1-X1-X2-B2-B3-X3-X4-B4,

where,

B1 - B3 = basic amino acid; and

X1 - X5 = alpha -helix enhancing amino acids.

ACTIVITY - Virucide; Anti-HIV; Hepatotropic; Antiinflammatory; Protozoacide.

Jurkat T-cells transduced with purified p16 fusion proteins were

infected by HIV and control cells transduced with vector not containing a HIV protease cleavage site. Result show efficient cleavage of p16 fusion proteins encoded by vectors containing HIV cleavage sites compared to control.

MECHANISM OF ACTION - Fusion protein (**cytotoxin**)-transduction **enhancer.**

USE - (I) is useful for treating pathogen infection in mammals, infections such as CMV, HSV-1, HCV, KSHV, yellow fever virus, flavivirus or rhinovirus, retroviral infections such as HIV-1, HIV-2, HTVL-3 and/or LAV, plasmodial infections associated with P.faciparum, P.vivax, P.ovale, P.malariae, cancer especially prostate cancer in which diseased cells express of property which can be targeted, such as elevated level of heavy metals e.g. zinc which promotes an inactive monomeric protein to become an active dimer. (I) is also useful for suppressing tumors by administering (I) comprising a cell cycle inhibitor such as p16, p27 or Cdk2DN along with a chemotherapeutic agent such as a DNA synthesis inhibitor that interacts in the S-phase of a targeted cell or a DNA damage initiator and thus promoting apoptosis (claimed).

ADVANTAGE - (D1) increases transduction efficiency of a protein by 5-10 fold and up to 100 fold as determined from intracellular concentrations of (D1) (claimed).

Dwg.0/21

L16 ANSWER 5 OF 8 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN

ACCESSION NUMBER: 2000-412091 [35] WPIDS
DOC. NO. CPI: C2000-124883
TITLE: Expression cassette used as live vector vaccine comprises nucleotide sequence encoding origin of replication and plasmid maintenance system which includes a post-segregational killing and a partitioning function.
DERWENT CLASS: B04 C06 D16
INVENTOR(S): GALEN, J E
PATENT ASSIGNEE(S): (UYMA-N) UNIV MARYLAND BALTIMORE
COUNTRY COUNT: 87
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2000032047	A1	20000608	(200035)*	EN	127
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW					
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US VN YU ZA ZW					
AU 2000020364	A	20000619	(200044)		
EP 1135025	A1	20010926	(200157)	EN	
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI					
NO 2001002721	A	20010731	(200157)		
CZ 2001001538	A3	20011114	(200175)		
HU 2001004609	A2	20020328	(200234)		
ZA 2001005383	A	20020424	(200237)		135
US 6413768	B1	20020702	(200248)		
MX 2001005449	A1	20011201	(200282)		
JP 2003506007	W	20030218	(200315)		170
US 6703233	B1	20040309	(200418)		
NZ 511449	A	20040528	(200437)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE

WO 2000032047	A1		WO 1999-US28499	19991202
AU 2000020364	A		AU 2000-20364	19991202
EP 1135025	A1		EP 1999-964042	19991202
			WO 1999-US28499	19991202
NO 2001002721	A		WO 1999-US28499	19991202
			NO 2001-2721	20010601
CZ 2001001538	A3		WO 1999-US28499	19991202
			CZ 2001-1538	19991202
HU 2001004609	A2		WO 1999-US28499	19991202
			HU 2001-4609	19991202
ZA 2001005383	A		ZA 2001-5383	20010629
US 6413768	B1		US 1998-204117	19981202
MX 2001005449	A1		MX 2001-5449	20010531
JP 2003506007	W		WO 1999-US28499	19991202
			JP 2000-584755	19991202
US 6703233	B1	CIP of Provisional	US 1998-204117	19981202
			US 1999-158738P	19991012
			US 1999-453313	19991202
NZ 511449	A		NZ 1999-511449	19991202
			WO 1999-US28499	19991202

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 2000020364	A	Based on	WO 2000032047
EP 1135025	A1	Based on	WO 2000032047
CZ 2001001538	A3	Based on	WO 2000032047
HU 2001004609	A2	Based on	WO 2000032047
JP 2003506007	W	Based on	WO 2000032047
US 6703233	B1	CIP of	US 6413768
NZ 511449	A	Div in	NZ 529508
		Based on	WO 2000032047

PRIORITY APPLN. INFO: US 1999-158738P 19991012; US 1998-204117 19981202; US 1999-453313 19991202

AN 2000-412091 [35] WPIDS

AB WO 200032047 A UPAB: 20000725

NOVELTY - An independently functioning expression cassette (I), comprises a nucleotide sequence encoding an origin of replication (ORI) and a nucleotide sequence encoding a plasmid maintenance system (PMS) which includes a post-segregational killing function (PSK) and a partitioning function.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) an amplifiable plasmid replicon (II) comprising (I);

(2) a bacterial cell comprising (II);

(3) an attenuated bacterial live vector vaccine (III), comprising a bacterial species containing a replicon comprising a nucleotide sequence encoding an antigen of interest, and a nucleotide sequence encoding a PMS;

(4) a conditionally unstable plasmid (IV), for examining changes in plasmid stability resulting from incorporation of plasmid maintenance system, comprises an ORI yielding an average copy number of 2-75 copies and a promoter driving the expression of a protein or peptide and whose over expression imposes a metabolic burden on a bacterium, which favors plasmid loss;

(5) making (M1) a stabilized (III), which involves transforming a bacterial live vector with a replicon comprising a PMS which includes one PSK and one partitioning function, and a nucleotide sequence encoding one or more antigen;

(6) a DNA (V), comprising a modified ompC promoter phenotypically characterized so that the promoter exhibits higher rates of osmotically

regulated expression in relation to a corresponding non-mutated ompC promoter; and

(7) an expression plasmid (VI) comprising (V).

ACTIVITY - Cytostatic; antibacterial; virucide; hepatropic; antiinflammatory; immunosuppressive; dermatological; antiasthmatic; antiallergic; neuroprotective; antiarthritic; antirheumatic; No supporting data is given.

MECHANISM OF ACTION - Vaccine.

USE - (IV) is used for eliciting an immune response in a human or bovine subject (claimed). (I) is used for transforming a bacterial cell which is cultured, and transformed into a subject to elicit an immune response. (I) can also be used to vaccinate a subject against Salmonella typhi. (I) may comprise an antigen for hepatitis B, Haemophilus influenzae type b, hepatitis A, acellular pertussis (acP), varicella, rotavirus, Streptococcus pneumoniae, or Neisseria meningitidis, and can be used as vaccines against diseases caused by these agents. (I) can be also used as a cancer vaccine. The antigens encoded by the plasmids are designed to provoke an immune response to autoantigens, B cell receptors and/or T cell receptors which are implicated in autoimmune or immunological diseases. Where an inappropriate immune response is raised against body tissues, or environmental antigens, the vaccines may immunize against the autoantigens, B cell receptors and/or T cell receptors to modulate the responses and ameliorate diseases, such as myasthenia gravis, lupus erythematosis, rheumatoid arthritis, multiple sclerosis, allergies and asthma.

ADVANTAGE - The plasmid maintenance systems incorporated into multicopy expression plasmids encoding one or more proteins or peptides of interest, enhances the level of expression of the protein or peptide of interest. The plasmid maintenance systems provide improved stability of recombinant plasmids, overcoming prior art problems of plasmid instability.

DESCRIPTION OF DRAWING(S) - The figure shows the pGEN expression plasmid pGEN2.

Dwg.1/8

L16 ANSWER 6 OF 8 MEDLINE on STN DUPLICATE 2

ACCESSION NUMBER: 1999003136 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9784530
TITLE: Disruption of an internal membrane-spanning region in **Shiga toxin** 1 reduces cytotoxicity.
AUTHOR: Suhan M L; Hovde C J
CORPORATE SOURCE: Department of Microbiology, Molecular Biology and Biochemistry, University of Idaho, Moscow, Idaho 83844, USA.
CONTRACT NUMBER: AI33981 (NIAID)
SOURCE: Infection and immunity, (1998 Nov) 66 (11) 5252-9. Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199811
ENTRY DATE: Entered STN: 19990106
Last Updated on STN: 19990106
Entered Medline: 19981123

AB **Shiga toxin** type 1 (Stx1) belongs to the Shiga family of bipartite AB toxins that inactivate eukaryotic 60S ribosomes. The A **subunit** of Stxs are N-glycosidases that share structural and functional features in their catalytic center and in an internal hydrophobic region that shows strong transmembrane propensity. Both features are conserved in ricin and other ribosomal inactivating proteins. During eukaryotic cell intoxication, holotoxin likely moves retrograde from the Golgi apparatus to the endoplasmic reticulum. The hydrophobic

region, spanning residues I224 through N241 in the Stx1 A **subunit** (Stx1A), was hypothesized to participate in toxin translocation across internal target cell membranes. The TMpred computer program was used to design a series of site-specific **mutations** in this hydrophobic region that disrupt transmembrane propensity to various degrees. **Mutations** were synthesized by PCR overlap extension and confirmed by DNA sequencing. Mutants StxAF226Y, A231D, G234E, and A231D-G234E and wild-type Stx1A were expressed in Escherichia coli SY327 and purified by dye-ligand affinity chromatography. All of the mutant toxins were similar to wild-type Stx1A in enzymatic activity, as determined by inhibition of cell-free protein synthesis, and in susceptibility to trypsin digestion. Purified mutant or wild-type Stx1A combined with Stx1B subunits in vitro to form a holotoxin, as determined by native polyacrylamide gel electrophoresis immunoblotting. StxA mutant A231D-G234E, predicted to abolish transmembrane propensity, was 225-fold less **cytotoxic** to cultured Vero cells than were the wild-type **toxin** and the other mutant **toxins** which retained some transmembrane **potential.** Furthermore, compared to wild-type Stx1A, A231D-G234E Stx1A was less able to interact with synthetic lipid vesicles, as determined by analysis of tryptophan fluorescence for each **toxin** in the presence of **increasing** concentrations of lipid membrane vesicles. These results provide evidence that this conserved internal hydrophobic motif contributes to Stx1 translocation in eukaryotic cells.

L16 ANSWER 7 OF 8 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 97269051 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9111051
TITLE: Role of the disulfide bond in **Shiga toxin** A-chain for toxin entry into cells.
AUTHOR: Garred O; Dubinina E; Polesskaya A; Olsnes S; Kozlov J; Sandvig K
CORPORATE SOURCE: Institute for Cancer Research at The Norwegian Radium Hospital, Montebello, 0310 Oslo, Norway.
SOURCE: Journal of biological chemistry, (1997 Apr 25) 272 (17) 11414-9.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199705
ENTRY DATE: Entered STN: 19970602
Last Updated on STN: 20000303
Entered Medline: 19970521

AB **Shiga toxin** consists of an enzymatically active A-chain and a pentameric binding **subunit.** The A-chain has a trypsin-sensitive region, and upon cleavage two disulfide bonded fragments, A1 and A2, are generated. To study the role of the disulfide bond, it was eliminated by **mutating** cysteine 242 to serine. In T47D cells this **mutated toxin** was **more toxic** than wild type **toxin** after a short incubation, whereas after longer incubation times wild type **toxin** was most **toxic.** Cells cleaved not only wild type but also **mutated** A-chain into A1 and A2 fragments. The **mutated** A-chain was **more** sensitive than wild type **toxin** to Pronase, and it was degraded at a higher rate in T47D cells. Subcellular fractionation demonstrated transport of both wild type and **mutated** toxin to the Golgi apparatus. Brefeldin A, which disrupts the Golgi apparatus, protected not only against **Shiga toxin** but also against the **mutated** toxin, indicating involvement of the Golgi apparatus. After prebinding of Shiga(C242S) toxin to wells coated with the **Shiga toxin** receptor, Gb3, trypsin treatment induced dissociation of A1 from the toxin-receptor complex demonstrating

that in addition to stabilizing the A-chain, the disulfide bond prevents dissociation of the A1 fragment from the toxin-receptor complex.

L16 ANSWER 8 OF 8 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 93350341 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8347933
TITLE: Entry of **Shiga toxin** into cells.
AUTHOR: Sandvig K; Dubinina E; Garred O; Prydz K; Kozlov J V; Hansen S H; Van Deurs B
CORPORATE SOURCE: Institute for Cancer Research, Norwegian Radium Hospital, Montebello, Oslo.
SOURCE: Zentralblatt fur Bakteriologie : international journal of medical microbiology, (1993 Apr) 278 (2-3) 296-305. Journal code: 9203851. ISSN: 0934-8840.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199309
ENTRY DATE: Entered STN: 19931001
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AB The effect of **Shiga toxin** with **mutations** in the A fragment has been tested on cells in order to get **more** information about the processing of the A fragment during entry into the cytosol. A mutant with a deletion between the A1 and A2 **domain** in the A fragment is resistant to cleavage by trypsin and is less toxic than wild type toxin on both Vero and A431 cells. The results support the view that processing of the A fragment is important for the high toxicity of the wild type toxin. A number of cell lines are resistant to **Shiga toxin** although they bind the toxin. However, A431 cells can be sensitized by butyric acid treatment, and transport of **Shiga toxin** to the Golgi apparatus seems to be required for the intoxication in the sensitized cells. The role of retrograde transport through the Golgi apparatus to the endoplasmic reticulum (ER) will be discussed.

=> FIL STNGUIDE

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	61.92	298.88

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
CA SUBSCRIBER PRICE	0.00	-0.74

FILE 'STNGUIDE' ENTERED AT 20:41:47 ON 28 JUL 2004

FILE CONTAINS CURRENT INFORMATION.
LAST RELOADED: Jul 23, 2004 (20040723/UP).

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=>
Executing the logoff script...

=> LOG H

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	0.18	299.06

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
CA SUBSCRIBER PRICE	0.00	-0.74

SESSION WILL BE HELD FOR 60 MINUTES
STN INTERNATIONAL SESSION SUSPENDED AT 20:43:21 ON 28 JUL 2004